as a unit in a ''knock-on'' mechanism of permeation. Differences in the amount of work required to move three Na^+ ions through the selectivity filter of NavAb compared to three K^+ ions predict the large negative reversal potentials observed for bacterial Nav channels in instantaneous current-voltage plots. The results of the simulations suggest that the block of bacterial voltage-gated Na^+ channels by extracellular K^+ does not occur in eukaryotic voltage-gated Na^+ channels because of differences in the amino acids present in the selectivity filters of the different channels.

2908-Pos Board B338

Molecular Dynamics Study of Ion Conduction and Selectivity in a Prokaryotic Ion Channel

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Since the publication of crystal structure of NavAb, the first of a (prokaryotic) voltage gated sodium channel, several computational studies have been aimed at determining the mechanisms of ion selectivity and ion conduction in voltage gated sodium channels. We provide a two-part study, well-converged free energy surfaces involving one, two and three ions provide results consistent with microsecond timescale simulations of ion conduction over concentration and voltages. The position of ions in the pore and cavity correlate to coordination number and side chain orientation, showing, as suggested by Chakrabarti et al. (PNAS (2013) 110, 11331). More surprising, presence of an ion in the aqueous cavity beneath the selectivity filter was sufficient to influence glutamate conformation.

Because the crystal structure of NavAb was in a closed pore conformation, we truncated the S5 and S6 helices and restrained these outer helices harmonically in a membrane represented by supporting lattice of neon. The turrets, pore helices and selectivity filter were free to move. Conductances were in the range of the experimental values; however, our studies show no preference for sodium conduction over potassium conduction at physiological conditions (−200mV, 0.15 M salt). While sodium conductance varied little with respect to concentration and presence of potassium in solution, stronger potassium selectivity is observed at 1 M and in mixed solutions. Our results are consistent with the observation of anomalous mole fraction effect in NaChBac by Finol-Urdaneta et al. (JGP (2014) 143, 157-171), though not sufficient to confirm such an effect. Additionally, we provide mechanisms for ion conduction showing a greater variety of states and transitions occupied during potassium conduction, and note that at higher concentrations of salt the mechanism of conduction is not as clearly cut.

2909-Pos Board B339

Coupling of Channel Fluctuations in Ion Permeation and Selectivity in Bacterial Sodium Channel NavAb

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Even though crystallographic structures of several cation channels are known at atomistic resolution, the molecular basis for selective ion permeation, and in particular, the role of structural fluctuations of the channel in that process, remains unclear. The determination of structures of voltage-gated sodium channels opens the way to elucidating the mechanism of sodium permeation and selectivity. Recent molecular simulation studies of bacterial sodium channel NavAb (Chakrabarti et al., PNAS 110, 11331-11336, 2013) suggest that Na^+ binding and permeation through the selectivity filter are coupled to the conformational isomerization of Glu177 side chains from an outfacing conformation to a lumen-facing conformation, resulting in a high rate of Na^+ diffusion through the selectivity filter.

To clarify the role of channel dynamics on ion permeation and selectivity, we examine the effect of structural constraints systematically. Specifically, we characterize the mechanism of cation permeation in the absence of conformational ''dunking'' of Glu177 side chains. In addition, we investigate the effect of structural restraints imposed on the pore helices to prevent channel closure, as well as of applied voltage, on channel fluctuations and transport properties. Results of simulations totaling over 100 microseconds indicate that restricting Glu177 conformations, either directly or through global structural restraints on the helices of the pore domain, modulates cation binding and permeation. Further, applying strong external voltage gradients significantly displaces the conformational equilibrium of the Glu177 side chains, thereby also modulating the mechanism of ion permeation in NavAb.

2910-Pos Board B340

Expression, Purification, and Preliminary Characterization of a Human Cardiac Sodium Channel Voltage Sensing Domain

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Voltage-sensing domains (VSDs) of voltage-gated ion channels sense changes in the membrane potential and as a result alter the conduction state of the channel. The human voltage-gated sodium channel NaV1.5 is primarily expressed in cardiac muscle and is responsible for the rising phase of the cardiac action potential. Mutations within NaV1.5 can lead to fatal cardiac arrhythmias. Such mutations have been found throughout the gene, including missense mutations within the VSD of repeat IV that have been shown to lead to Brugada Syndrome and LQT3. The VSDs also play an important role as binding sites for gating modifier peptide toxins from tarantula spider venoms. Such toxins could serve as good lead compounds for drug development due to their high specificity and more subtle mode of action compared to pore blockers. Therefore, it would be important to know the structures of the human sodium channels VSDs. While no structures of whole eukaryotic sodium channel proteins exist, isolated VSDs of other ion channels have been shown to fold into their native conformation in the absence of the pore forming domain. Therefore, we are pursuing the expression and purification of the isolated VSDs of human sodium channels in order to investigate the structural changes within the VSD caused by pathogenic mutations and by the binding of gating-modifier toxins. Here, we present the expression and purification of the human NaV1.5 VSD of repeat IV in a bacterial expression system in isotopically labeled form and preliminary characterization of the truncated protein. NaV1.5 VSD IV is expressed in Escherichia coli in minimal media, and extracted from membranes by solubilization into n-dodecylphosphocholine micelles. Purified NaV1.5 VSD IV was characterized by mass spectrometry and gel filtration chromatography and used for preliminary NMR structural studies.

2911-Pos Board B341

A Thermodynamic Analysis of Disease-Causing Mutations in the Nav1.5 C-Terminus

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The opening of voltage-gated sodium channels (Na_v) is responsible for the rapid upstroke of action potentials. A key player during myocardial excitation is the cardiac channel isoform $Na_v1.5$. The general architecture of mammalian Na_vs is comprised of four homologous domains, containing six transmembrane segments each, and a C-terminal intracellular domain (CTD) carrying an IQ-motif. The individual domains are connected by large intracellular linkers. The linker connecting domain three and four as well as the CTD seem to play a role in channel inactivation which is different from regular channel closing but poses an important function to modulate ion conductance. The rapid inactivation of channels limits the influx of ions and therefore depolarization of the cell per opening signal. In this context the CTD is of particular interest as an interaction partner for regulatory proteins as calmodulin (CaM) as well as hotspot for disease-causing mutations that have a profound influence on channel inactivation. To elucidate the functional effects of disease-causing mutations we expressed mutant channels in *Xenopuslaevis* oocytes and studied them by two-electrode voltage clamp. To complement the data we analyzed the thermostability of isolated mutant CTDs and performed isothermal titration calorimetry experiments. Isothermal titration calorimetry experiments in the absence and presence of Ca^{2+} were used to determine binding profiles of individual CaM lobes to WT and mutant CTDs. Our data shows that mutations have distinct effects on the folding stability and ability to bind CaM. Whereas some mutations cause misfolding of the CTD, others selectively affect binding of apoCaM or both apoCaM and Ca^{2+}/CaM, and these changes correlate with the disease phenotype.

2912-Pos Board B342

Functional Consequences of a Novel Nav1.9 Mutation (L1302F) causing Congenital Insensitivity to Pain

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The contribution of the peripheral nerve voltage-gated sodium (Na_V) channel $Na_V1.9$ to nociception has been demonstrated in $Na_V1.9$ knockout mice that

have greatly reduced inflammatory pain sensation. More recently, mutations in *SCN11A* encoding human $Na_V1.9$ have been associated with either loss of pain perception, or familial episodic pain and painful peripheral neuropathy. Here we elucidate the functional consequences of a novel heterozygous $Na_V1.9$ mutation (L1302F) discovered in a female diagnosed with congenital insensitivity to pain (CIP). The mutation was stably expressed in ND7/23 cells and whole-cell currents were elicited with 50 ms pulses from −120 to +40 mV from a holding potential of −140 mV in the continuous presence of 150 nM TTX to block endogenous TTX-sensitive sodium currents. Cells expressing $Na_V1.9$-WT exhibited whole-cell current that peaked at −40 mV (31.2 ± 3.5 pA/pF, n=10) with a voltage-dependence of activation defined by V½ = −61.8 ± 1.5 mV and slope factor (k) of 6.1 ± 0.4 (n=10). By contrast, $Na_V1.9$-L1302F whole-cell current peaked at −70 mV (39.5 ± 8.7 pA/pF, n=12) and exhibited a significantly hyperpolarized voltage-dependence of activation (V½ = −86.6 ± 1.1 mV; k = 6.5 ± 0.4; n=12). These results initially appeared to indicate that the mutation potentiates channel function by enabling activation at more negative membrane potentials. However, when currents were recorded using a holding potential of −90 mV, mutant channel activity was reduced substantially by ~95% (1.5 ± 0.3 pA/pF @ −70 mV, n=7), whereas the WT channel had more preserved activity ~50% (15.6 ± 3.1 pA/pF @ −40 mV, n=6). These results suggest that the effect of this mutation is more likely a loss-of-function under physiological conditions, and this will reduce neuronal excitability leading to impaired pain sensation.

2913-Pos Board B343

Infant Sudden Death: Novel Mutations Responsible for Impaired Nav1.5 Channel Function

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Sudden infant death syndrome (SIDS) is the leading cause of mortality in apparently normal infants. During 2008 to 2012, the New York City Office of Chief Medical Examiner (OCME) examined 274 cases of sudden unexplained death (SUD) of which 141 were infants below 1 year of age, with ~93% of these less than 6 months of age at the time of death. Several ion channelopathies were found during genetic screening. An African-American/Hispanic girl who died suddenly in her sleep at the age of 5 weeks carried two SCN5A mutations: c.5494 C>G and c.5830 C>T, which respectively introduces a missense mutation Q1832E and an early stop codon R1944X in the distal C-terminus of the cardiac Na^+ channel -subunit, Nav1.5. HEK-293 cells were transfected with cDNAs of wild-type Nav1.5, Nav1.5-Q1832E, Nav1.5Δ1944 (the C-terminal truncation) or a cDNA with both mutations (Nav1.5-Q1832E-Δ1944) and were subjected to whole-cell patch clamping. The peak Nav1.5-Q1832E current was reduced by almost 10-fold (e.g. at −20mV the wild-type Nav1.5 was 283 ± 49.1 pA/pF, n=8 cf. −31 ± 11.8 pA/pF, n=4, for Nav1.5-Q1832E, $p<0.001$), whereas Nav1.5Δ1944 and Nav1.5-Q1832E-Δ1944 currents were not significantly different from wild-type. The inactivation time constants were unaffected by any of the mutations (e.g. at −10mV, 1 and 2 respectively were 1.3 ± 0.15 and 7.7 ± 0.87 ms, n=8 for wild-type cf. 1.1 ± 0.21 and 7.5 ± 1.61 ms, n=4, for Nav1.5-Q1832E). No significant differences were observed for the time course of the recovery from inactivation or the voltage dependence of the activation and inactivation kinetic variables. Preliminary biotinylation experiments suggest that the Nav1.5-Q1832E surface expression was unaltered compared to wild-type, suggesting a defect independent of trafficking. In conclusion, the Q1832E mutation was sufficient to produce a severely dysfunctional Nav1.5 channel, which may have been contributing to the victim's sudden death.

2914-Pos Board B344

Biophysical and Molecular Analysis of the Sodium Current in Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes

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Background: Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have been used for safety pharmacology and to investigate genetic diseases affecting cardiac ion channels. We examined I_{Na} in hiPSC-CMs and determined its contribution to action potentials (APs) recorded from monolayers of hiPSC-CMs.
Methods: Commercially available hiPSC-CMs were plated at high density to form monolayers or low density to yield single cells. AP recordings from monolayers were made using high resistance electrodes at 36°C. Whole cell patch clamp was used to record I_{Na} in single hiPSC-CMs at room temperature.
Results: AP recordings showed spontaneous activity with a maximum diastolic potential (MDP)= −69.2 ± 1.4 mV and upstroke velocity=41.9 ± 6.7 V/s. Application of tetrodotoxin resulted in a slowing of the AP rate but had little effect on AP upstroke or duration. In single hiPSC-CMs, a large I_{Na} was recorded when external Na^+ was reduced to 40 mM (73.6 ± 6.18 pA/pF). Recovery of I_{Na} (hp= 120 mV) was very fast; at hp= 80 mV, recovery of I_{Na} was slower and the size of peak I_{Na} was greatly reduced (27.0 ± 3.38 pA/pF). Molecular analysis showed that SCN5A was the predominate Na^+ channel subtype in both adult and iPSC-CMs. In adddition, we found that iPSC-CMs express both the fetal (exon 6A) and adult (exon 6) isoforms of SCN5A. Action potential clamp experiments showed that application of a ventricular or Purkinje cell waveform to the same hiPSC-CM elicited a large I_{Na} while application of a SA node waveform elicited no I_{Na}.
Conclusion: A large I_{Na} is present in hiPSC-CM but its contribution to the AP upstroke is minimal. The depolarized MDP coupled with the presence of phase 4 depolarization results in a take-off potential of −60.6 ± 1.7 mV which inactivates the majority of Na^+ channels.

2915-Pos Board B345

Nav1.5 C-Terminal Domains Influence Calcium Regulation of Fast Inactivation Separately from Calmodulin Interaction

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The cardiac sodium channel (Nav1.5) has a complex 'intracellular Ca^{2+} sensing apparatus' within its C-terminal domain (CTD) consisting of a partial EF-hand domain (CTD-EF) and a calmodulin (CaM) binding IQ motif (CTD-IQ). There are additional CaM binding motifs within the DIII-DIV linker. Variation in intracellular Ca^{2+} concentration influences the voltage-dependence of steady-state fast inactivation (SSI) by an unclear mechanism. Here, we mutated 16 key residues implicated in the interactions between CTD-EF and CTD-IQ, CTD-IQ and CaM, or DIII-DIV and CaM and explored their impact on NaV1.5 function and biochemistry. We used quantitative yeast-two-hybrid assays to measure effects of mutations on the interaction between the full length CTD on CaM, and evaluated SSI in high (1 μM free Ca^{2+}) and low $[Ca^{2+}]$i conditions. Using either BAPTA or HEDTA as chelator, 1 μM free $[Ca^{2+}]$i was sufficient to shift SSI (elicited by 50 ms prepulses) towards depolarized potentials. We observed that 3 of 5 CTD-IQ mutations (F1912A, A1924T and IQ/AA) strongly reduced the interaction between the CTD and CaM. However, these mutations did not affect the $[Ca^{2+}]$i effect on SSI suggesting that SSI $[Ca^{2+}]$i sensitivity does not depend on CaM binding to the CTD-IQ. Unexpectedly, single and combination mutations of the CTD-EF (L1786A, F1791A, Q1807A, L1862A, E1788A-D1790A-D1792A-E1799A, E1804A-D1802A) diminished the CTD-CaM interaction, and some of these mutations (L1786A, E1788A-D1790A-D1792A-E1799A, E1804A-D1802A) also suppressed the $[Ca^{2+}]$i effect on SSI. Indeed, the only mutations we studied that blunted the $[Ca^{2+}]$i effect on SSI were within the CTD-EF. These results suggest that the CTD-EF influences NaV1.5 $[Ca^{2+}]$i sensitivity and mutations in this domain can also alter the interaction of the CTD with CaM, but CaM interaction with the CTD-IQ is not required for the effect of intracellular Ca^{2+} on inactivation.

2916-Pos Board B346

CaMKII-Dependent Regulation of Cardiac Sodium Channel

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Voltage-gated Na^+ channels are key determinants of conduction, action potential profile and refractoriness in mammalian myocardium. Na^+ channels are regulated by a number of protein kinases and alterations in phosphorylation are associated with the phenotypic expression of inherited and acquired heart diseases. Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) phosphorylates $Na_V1.5$ at multiple sites in the domain I-II linker, with effects on channel gating. The CaMKII phosphorylated sites we identified by mass spectrometry (MS) include serines 459, 460, 484, 539, 571, 664, 667 and threonine 486 in the I-II linker and serines 1925, 1937 and 1969 in the carboxyl-Terminus (CT). In addition we evaluated the effect of CAMKII phosphorylation on $Na_V1.5$-S528A and $Na_V1.5$-R526H channels in which direct phosphorylation by PKA is abolished (Aiba, 2014). In Wild-type $Na_V1.5$ channels, acute exposure to CaMKII increased the peak current,